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A Shortcut to Activity-Dependent Transcription

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Neuronal activity results in the rapid induction of gene transcription through a series of defined molecular events. Madabhushi et al. describe an unexpected role for the cutting of promoter DNA by topoisomerase IIB to facilitate transcription of activity-induced genes.

Sensory experience induces activity-dependent gene expression in neurons, and this process has been implicated in the function and dysfunction of the nervous system (West and Greenberg, 2011). The ability of a neuron to rapidly induce gene transcription in response to sensory stimuli requires the binding of pre-existing transcription factors such as CREB, SRF, and MEF2 to promoters and enhancers. In response to external stimuli, these factors become modified, typically by phosphorylation or dephosphorylation, leading to enhancer and promoter engagement. This is followed by the release of paused, promoter-bound RNA polymerase II (RNAPII) complexes resulting in productive transcriptional elongation (Figure 1A). Prior to neuronal activity, a time when activity-dependent genes are expressed at low levels, these genes express hallmarks of highly expressed genes (e.g., binding by transcription factors and polymerase, and trimethylation of Histone H3 at lysine 4 at promoters). This suggests that activity-dependent genes are poised for activation but that a switch or set of switches must be flipped in order for transcriptional activation to occur.

In this issue of *Cell*, Madabhushi et al. (2015) propose that some activity-regulated genes are maintained in a state of high torsional stress prior to stimulation such that supercoiling of the DNA keeps RNAPII from extending into gene bodies. The authors provide evidence that upon neuronal depolarization, activation of Topoisomerase IIB (Topo IIB) leads to DNA double-stranded breaks (DSBs) within the promoters, thus allowing the DNA to unwind and RNAPII to productively elongate through gene bodies (Figure 1B).

DSBs have classically been viewed as unwanted DNA damage and have been linked to pathological states including neurological disorders (Madabhushi et al., 2014). However, recent reports have noted that neuronal stimulation leads to the appearance of hallmarks of DSBs in the nucleus of neurons (Suberbielle et al., 2013), including the phosphorylation of serine 139 on the histone variant H2AX (γH2AX), a chromatin mark deposited on adjacent histones by the DNA-damage response pathway immediately after DSBs are detected. This suggested that DSBs might occur as part of the normal cellular response to neuronal activation, but where on the genome these DSBs occur, and what function they play, has remained unclear.

Madabhushi et al. sought to understand the effects of DSBs by inducing them in neurons using a topoisomerase inhibitor drug, etoposide, and investigating the effects on gene expression by RNA-sequencing (RNA-seq) analysis. Etoposide traps type II topoisomerase enzymes (those that make double-stranded cuts in the DNA) in a state where they remain bound to cleaved DNA, and this subsequently can lead to the formation of DSBs. Upon etoposide treatment, the authors observe an increase in transcription at several genes, including *Fos*, *FosB*, and *Npas4*, all of which are known to be rapidly transcribed in response to neuronal activity. Inhibition of the most prevalent type II topoisomerase in neurons (TopIIB) by RNAi knockdown conversely leads to blunted induction of these genes, suggesting that the cutting of DNA by TopIIB is essential for full gene activation.

To further explore if activity-dependent gene induction is linked to DSB formation, the authors examine the distribution of γH2AX in activated neurons by chromatin immunoprecipitation followed

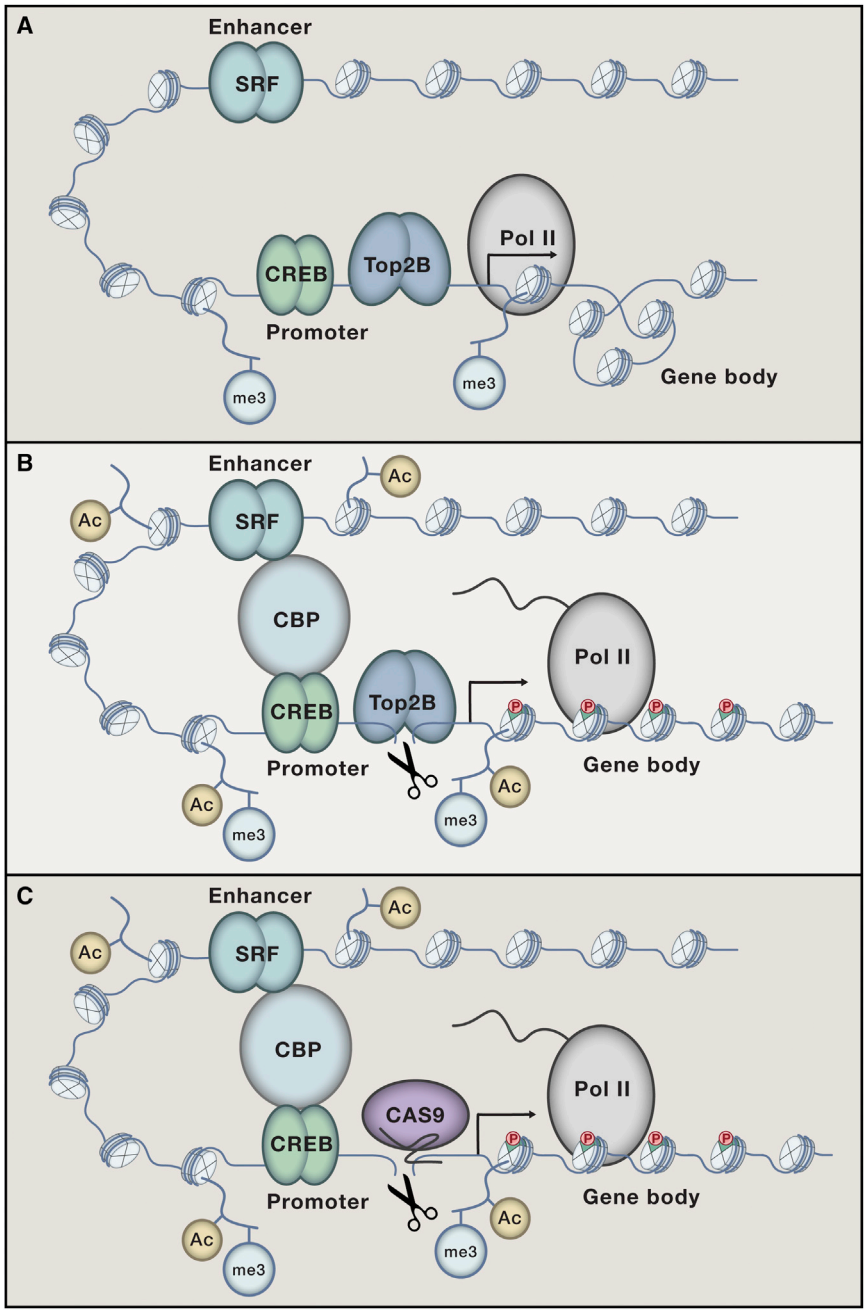

Figure 1. Topoisomerase Induces DNA Double-Strand Breaks at Activity-Regulated Genes
(A–C) Genes that are rapidly induced in response to neuronal stimulation (e.g., *Fos*) display hallmarks of active transcription before induction, including transcription factor binding, the accumulation of H3K4me3, and paused RNA polymerase II at the transcription start site (A). Upon neuronal stimulation, recruitment of coactivators (e.g., CBP) to enhancers and promoters leads to histone acetylation and induction of transcription (B). Madabhushi et al. present evidence that Toposimerase IIB (Top IIB) binds to the promoters of these genes and that topoisomerase-dependent formation of double-stranded breaks (DSB) relieves topological constraints to facilitate transcriptional activation. A build-up of a DSB-associated histone mark (phosphorylation of H2AX at Ser 139) occurs throughout the transcribed regions of these activated loci. Depletion of Top IIB in neurons leads to impaired induction of activity-dependent genes, but the targeting of the Cas9 nuclease to the promoters of these genes can reverse this effect (C), supporting a role for DSBs in activity-dependent gene activation.

by sequencing (ChIP-seq). γH2AX is known to initiate and rapidly spread from the site of damage in response to DSBs (Álvarez-Quilón et al., 2014). The authors observe that ChIP-seq signal for γH2AX increases after neuronal activity throughout the transcribed region of immediate early genes that are upregulated in response to etoposide treatment or upon treatment with agents that lead to membrane depolarization indicating that at least part of the DNA-damage response pathway is triggered by transcription-associated DSB formation.

Madabhushi et al. hypothesize that if the formation of DSBs relieves torsional stress and is critical to the induction of activity-regulated genes, then artificially introducing DSBs should relieve topological stress and create a permissive environment for RNAPII to enter productive elongation. To test this directly, the authors synthetically introduce DSBs at target promoters using CRISPR/Cas genome editing system. Consistent with their model, when DSBs are targeted to the *Fos* promoter by CRISPR/Cas, a significant increase in transcription of the *Fos* locus is observed, even in the absence of TopIIB (Figure 1C). These observations led the authors to propose that neuronal activity induces the TopIIB-dependent formation of DSBs at activity-dependent genes to relieve topological constraints and facilitate transcription.

The formation of DSBs resulting from of topoisomerase activity during the normal induction of gene expression represents a noncanonical role for topoisomerases in transcription. Classically, the cutting, unwinding, and re-ligation of DNA by topoisomerases is thought to occur efficiently, without substantial formation or perdurance of DSBs that could trigger DNA-damage response pathways and cause the build-up of gamma-H2AX (Smeenk and van Attikum, 2013). Rare aborted topoisomerase reactions can result in DSBs, but these are thought to be errors that are rapidly corrected through the activity of tyrosyl-DNA phosphodiesterase (TDP) enzymes and the nonhomologous end joining (NHEJ) pathway. However, in the current study Madabhushi et al. provide evidence of the formation of DSBs and also implicate the activity of TDP enzymes and NHEJ

in the normal induction of activity-induced genes. This suggests that topoisomerase-dependent DSBs may not just be a rare unwanted occurrence, but may also play a regular, important role in the normal activity-dependent transcription.

One might note that the repeated formation of DSB followed by NHEJ at activity-dependent loci appears to be a risky strategy for a neuron. If DSBs form at activity-induced genes each time the neuron is activated, over the course of the life of an organism, even a low error rate in the repair process would lead to a significant mutational load. When combined with the previous finding that there is an increase gamma-H2AX phosphorylation in vivo as a consequence of sensory experience (Suberbielle et al., 2013), the results of Madabhushi et al. raise the possibility that a negative consequence of the normal activation of neurons may be a high rate of mutation at activity-induced genes. As the authors note, this mutagenesis might cause genome instability, or be disruptive to activity-dependent gene expression programs later in the life of an organism. Furthermore, the recent observation that increased activity-induced gamma-H2AX is observed in activated neurons of Alzheimer's mouse models (Suberbielle et al., 2013), suggests that this pathway could contribute to DNA damage in neurodegenerative disease. Future studies examining if extensive mutagenesis occurs specifically at regulatory regions of activity-dependent genes in the aging brain will help to test this prediction.

These new findings by Madabhushi et al. add to a growing body of evidence that topoisomerase pathways may play a particularly important role in transcriptional regulation in the brain. Recent studies have demonstrated the critical role of TDP enzymes in normal brain development and function (Gómez-Herreros et al., 2014) and underscore the importance of topoisomerase activity in facilitating the expression of very long genes (King et al., 2013), which are critical to the function of the brain (Gabel et al., 2015). Taken together these findings indicate that topoisomerase function is central to the development and long-term health of the mammalian brain, and that further study of this key group of enzymes has the potential to give important new insight into brain plasticity and disease.

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Flies Sleep on It, or Fuhgeddaboudit!

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Many studies in diverse organisms, including humans, have demonstrated a fundamental role for sleep in the formation of memories. A new study by Berry et al. indicates that, in fruit flies, sleep accomplishes this in part by preventing an active process of forgetting.

> Our birth is but a sleep and a forgetting.—William Wordsworth (*Ode: Intimations of Immortality*)

Anyone who has crammed for an exam will tell you that memorizing takes considerable effort, whereas forgetting happens all too easily. In actuality, forgetting is a regulated mechanism in the brain for discarding useless information in favor of storing more salient memories. Recent work in *Drosophila* has emphasized that the forgetting of memories formed during aversive olfactory conditioning is an active process of the brain, with molecular and neuronal substrates that are distinct from the processes that regulate memory formation (Berry and Davis, 2014). In this issue of *Cell*, Berry et al. (2015) extend these observations to show that sleep results in better memory retention by disabling a key "forgetting circuit" in the *Drosophila* brain that is normally active during arousal.